Cytogenetic Analysis of 65 Women with Premature Ovarian Insufficiency

Prematür Over Yetmezliği Tanısı Alan 65 Kadında Genetik Analiz Sonuçları

Genetic Analysis in Women with Premature Ovarian Failure

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Özet

Amaç: Prematür over yetmezliği (POY) 40 yaşından önce gözlenen, 6 ay veya daha uzun süre adet görememe, serum folikül stimüle edici hormon yüksekliği ve düşük serum estradiol düzeyi ile tanımlanan bir durumdur. Bu çalışmada amaç POY tanısı alan olgularda kromozom anomali tipleri ve sıklığı araştırmaktır. Gereç ve Yöntem: İdiopatik POY tanısı ile Bezmialem Vakıf Üniversite Hastanesi Tıbbi Genetik Anabilim Dalı'na yönlendirilen 65 olgunun karyotip analiz kayıtlarının retrospektif olarak incelenmesiyle yapılmıştır. Bulgular: 65 olgunun 12'sinde (%18,4) kromozom anomalisi saptandı. Tüm olgularda sayısal X kromozom anomalisi mevcuttu. En sık saptanan kromozom anomalisi X kromozom mosaismi idi. 2 olgu, FMR1 gen premutasyon taşıyıcısı idi. Ailesel POY 8 olguda (%12,3) oranında saptanmıştır. Tartışma: Bu çalışma POY etiyolojisinde X kromozom anomalilerinin önemini vurgulamıştır. POY yönetiminde sitogenetik incelemenin klinik özelliklere ve yaşa bakılmaksızın rutine girmesi gerekmektedir.

Anahtar Kelimeler

Prematür Over Yetmezliği; X Kromozom Anomalisi; Karyotip; Mosaism

Abstract

Aim: Premature ovarian insufficiency (POI) is characterized as amenorrhea for more than 6 months, occurring before the age of 40, with an increased follicle-stimulating hormone and low estrogen concentrations. The aim of our study is to determine the types and distribution of cytogenetic abnormalities among women with POI. Material and Method: The study is based on the retrospective karyotype analysis of 65 women with idiopathic POI referred to the Medical Genetics Department at the Bezmialem Vakif University Hospital. Results: Chromosomal abnormalities were present in 12 of 65 cases (18.4%). All of them had numerical abnormalities of the X chromosome. The most frequently detected abnormalities were X chromosome mosaicisms. Two cases had fragile X premutation carriers. Eight (12.3%) women were considered as familial POI. Discussion: Our results underline the essential role of the X chromosome in the etiology of POI. Therefore, regardless of clinical features and woman's age, cytogenetic investigations should be routinely performed in cases with POI.

Keywords

Premature Ovarian Insufficiency; X Chromosome Abnormalities; Karyotype; Mosaicism

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Introduction

Premature ovarian insufficiency (POI) is characterized by amenorrhea, the presence of high levels of gonadotropins [follicle-stimulating hormone (FSH) > 40 mIU/ml], and low levels of estradiol (E2) for at least 6 months duration in women before the age of 40 [1]. POI occurs in ~1% of the general female population before 40 years of age, as its incidence according to age is approximately 10/100,000 in women aged 15 to 29 years and 79/100,000 in women aged 30 to 39 years [2]. The prevalence of familial POI has been reported as 12.5% to 50% with widely varying percentages in series [2-4].

While the etiological causes of (POI) are highly heterogeneous, most cases of isolated POI still appear sporadically. The demonstrated etiological causes of POI include chromosomal or genetic (premutation of the FMR1, either numerical or structural abnormalities of an X chromosome such as monosomy, trisomy, deletions, translocations or autosomes), autoimmune, metabolic (such as galactose-1-phosphate uridyltransferase), infectious (e.g. viral), and iatrogenic factors (e.g. extensive pelvic surgery or oophorectomy, radiation, and chemotherapy) [5]. The importance of genetics in the etiology of POI is supported by the observation that POI occurs in approximately 10%-30% of idiopathic cases [6]. The prevalence of the FMR1 premutation carrier state in the general population of women is 1 in 100 and approximately 16-26% of the female premutation carriers will develop POI [7]. Autoimmunity is responsible for approximately 4-30% of POI cases [8].

The aim of this study is to investigate the prevalence and type of cytogenetic anomalies in 65 Turkish women with POI in order to assess the efficacy of cytogenetic screening.

Material and Method

This study was based on a review of medical records of the karyotype analysis of 65 women with POI who were referred by clinicians to the Medical Genetics Department of the Bezmialem Vakif University Hospital from November 2011 to December 2013. All medical and family histories of the women were taken by gynecology and genetic clinics. Inclusion criteria included secondary amenorrhea for at least 4 months prior to the age of 40 years and two serum FSH measures higher than 40 mIU/ml obtained at least 1 month apart. Exclusion criteria were conditions that can induce POI, such as ovarian surgery, chemo- or radiotherapy, or autoimmune diseases. This study was approved by the Ethics Committee of our hospital and informed consent was obtained from all participants.

Collected data included medical history, menstrual cycle irregularities, menopause age, obstetric history (gravidity, parity, a history of previous miscarriage, and curettage), and measurement of height and weight. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m2). Positive family history was considered if another first- or second-degree relative had POI. POI was defined as familial when the index woman had at least two family members also affected by POI.

Serum samples were collected in a fasting state for the measurement of serum FSH and E2 level. Serum FSH concentrations were measured by a direct chemiluminescence immunoassay and E2 was determined by a competitive chemiluminescent immunoassay (ADVIA Centaur, Siemens Healthcare Diagnostics, NY, USA). Metaphase chromosomes prepared by standard cytogenetic methods were analysed from the collected peripheral blood samples. Twenty metaphase spreads per patient were routinely banded using the trypsin Giemsa technique at resolutions of 500 to 650 bands per haploid set. After evaluation, 100 spreads were additionally banded in cases with mosaic mutations. DNA testing for fragile X premutation was also done.

DNA samples were collected from peripheral blood samples. The number of CGG repeats in the FMR1 gene located on XQ27.3 was analysed with direct fluorescent PCR and capillary electrophoresis fragment analysis. NM_002024.3 was considered as the reference point. The number of CGG repeats 5-40, 41-58, 59-20, and >200 were respectively defined as normal, intermediate allel with risk of mutation, premutant allel, and complete expansion.

Analyses were done using the Statistical Package for the Social Sciences, version 21 (SPSS, Chicago, IL). Data were expressed as mean ± standard deviation or number and percentage, as appropriate.

Results

The results of karyotype analyses of four patients were not found. The remaining 65 patients admitted with secondary amenorrhea were included in the present study. Demographic characteristics and hormone profiles of patients are summarized in Table 1. The average age was 36.38 ± 4.63 at the time of cytogenetic exploration. Of 24 cases (36.9%), at least one first- or second-degree relative had POI and in 8 cases (12.3%) familial POI was identified.

The karyotype was detected as normal in 51 cases (78.4%) and the frequency of genetic abnormalities was 21.5% (14 cases) in patients with POI. Numerical X chromosome abnormalities (12 cases; 18.4%) were the most common abnormalities detected in women with POI in our study. Two cases were fragile X premutation carriers. We also detected 1 case of 46,XX,15p+. The distribution of abnormalities in patients with POI is detailed in Table 2.

All patients had a complete physical examination at the first visit. There was no patient with abnormal secondary sexual characteristics or with characteristic features of Turner syndrome such as short stature, webbed neck, hypoplastic uterus, or other dysmorphic features. The anti-thyroid peroxidase antibody was detected in only a case with 45,X[5] /47,XXX[1] /46,XX[94] . The patient with fragile X premutation carrier was mentally normal but she had a mentally impaired son diagnosed with fragile X syndrome. We did not detect any kinds of structural X chromosome abnormalities and autosomal anomalies in patients with POI.

Discussion

The etiological causes of POI are clearly heterogeneous, with a wide spectrum of causes, and in most cases its underlying mechanisms remain unknown. Structural and numerical abnormalities of the X chromosome were the most common genetic causes of POI because two intact X chromosomes are necessary for normal development of the ovary and of ovarian function. Cytogenetic analysis is the tool currently available for the detection of cytogenetic abnormalities leading to POI.

POI is defined as familial when an index woman with POI has at least two relatives with POI [9]. Several studies reported an incidence of familial POI of between 4% and 33% [3,10]. On the other hand, a study that included 200 cases having at least ≥1 relative with POI found that the incidence of familial POI was 29% [4]. According to a study from Turkey, the incidence of familial POI was reported as 24% [9].

In a recent study including 175 cases, Geckinli et al. [11] detected chromosomal abnormalities in 35 of 94 (37%) cases with primary amenorrhea and in 9 of 81(11.1%) cases with POI. Furthermore, X-aneuploidy or X-structural abnormalities or 46,XY karyotype were the most frequently detected abnormalities in this study. When compared to our results, some studies reported higher frequency while others reported lower frequency of chromosomal abnormalities. The prevalence of chromosomal abnormalities reported in previous studies is presented in Table 3 [1,4,12-18]. The different prevalence in these studies probably results from selection biases because women with primary amenorrhea were included for analysis in some of the studies from different countries.

Table 1. Characteristics of 65 women with POI.

Age at diagnosis (years)	36.38 ± 4.63
Age of amenorrhea (years)	2.23 ± 2
BMI (kg/m²)	25.79 ± 4.10
Gravidity	2.12 ± 1.81
Parity	1.56 ± 1.10
History of miscarriage	0.39 ± 1.32
History of curettage	0.18 ± 0.57
Plasma FSH concentration (mIU/ml)	72.70 ± 31.04
Plasma estradiol concentration (pg/ml)	36.15 ± 31.65

Data are shown as mean ± standard deviation.
POI, premature ovarian insufficiency FSH: follicle-stimulating hormone.

Table 2. Prevalence and distribution of karyotype abnormalities in women with POI.

Character of the disorder	No. of cases (%)
X-numerical abnormalities	12 (18.4)
45,X[3] /47,XXX[1] /46,XX[96]	3 (4.6)
45,X[1] /47,XXX[2] /46,XX[97]	1 (1.5)
45,X[7] /47,XXX[1] /46,XX[92]	1 (1.5)
45,X[2] /47,XXX[3] /46,XX[95]	1 (1.5)
45,X[5] /47,XXX[1] /46,XX[94]	1 (1.5)
45,X[3] /47,XXX[2] /46,XX[95]	1 (1.5)
45,X[2] /47,XXX[2] /46,XX[96]	1 (1.5)
45,X[5] /46,XX[95]	2 (3)
45,X[2] /46,XX[98]	1 (1.5)
Fragile X premutation carrier	2 (3)

The strong relationship between X chromosome and ovarian function and the etiology of POI is commonly highlighted in the literature [5,13]. A Dutch study also indicated the relationship between X chromosome and the broader spectrum of menopausal age and POI [19]. However, the present study found that the most frequently detected abnormalities were numerical abnormalities of the X chromosome involving X chromosome mosaicisms. A study reported that the numerical chromosomal abnormalities were 8% of the patients with POI consisting of 4 X chromosome mosaicisms and 2 X chromosome trisomies [9]. Wu et al. [20] found X chromosome mosaicism in 5 (8.2%) of the 65 women with POI in their study. Our results are in accordance with those reported by Ayed et al. [21]. They reported mosaicism with a 45,X line as the most frequently detected anomaly in women with POI and also reported that the prevalence of 47,XXX in 100 POI cases was 3%, including two mosaic and one non-mosaic. There is no clear evidence to indicate the effect of an X trisomy on human fertility, as the strong relationship between an X trisomy and POI is clearly revealed [22]. In fact, our data may support that women with X chromosome mosaicism may experience premature menopause [13,20]. The prevalence of 47,XXX in cases with mosaic pattern was 10.7 %, which is higher than the prevalence reported by Goswami et al. [5] (3.8%) and Jiao et al. [12] (1.5%), respectively.

Autosomal chromosomal abnormalities are uncommon in women presenting with amenorrhea. We detected only 1 case of 46,XX,15p+ which is phenotypically normal. In a more recent study, Demirhan et al. [23] analysed chromosome abnormalities in 393 women presenting with primary and secondary amenorrhea and 46,XX,15p+ was found in 1 case. As the p arm of acrocentric chromosomes has no gene, it is expected that the increasing heterochromatin material in the p arm of acrocentric chromosomes considered as a polymorphic feature does not influence phenotype.

Varying data have been published regarding the incidence of FMR1 premutation carrier in women with POI. Murray et al. [24] showed that the ratio of FMR1 premutation carrier was 1.6% in sporadic cases and 16% in familial POI cases and they suggest that patients with idiopathic POI should be routinely screened for fragile X. Bachelot et al. [25] reported the incidence of FMR1 premutation carrier was 7% in the familial cases and 8% in

Table 3. Summary of frequency of chromosomal abnormalities in different population studies of POI.

	Frequency of CA (%)	No. of CA	Sample size	Clinical characteristics	Population	References
Present study	18.4	13	65	POI	Turkish	Our study
Ayed et al. (2014)	18	18	100	PA, SA	Tunisian	[21]
Geçkinli et al. (2014)	25	44	175	PA, POI	Turkish	[11]
Kalantari et al. (2013)	10.05	18	179	PA, SA	Iranian	[14]
Jiao ve et al. (2012)	12.1	64	531	PA, SA	Chinese	[12]
Baronchelli et al. (2011)	10	27	269	PA, SA, EM	Italian	[15]
Lakhal et al. (2010)	10.8	108	1000	PA, SA	Tunisian	[13]
Ceylaner et al. (2010)	25.3	19a	75	SA	Turkish	[9]
Janse et al. (2010)	12.9	19	147	SA	Dutch	[4]
Portnoi et al. (2006)	8.8	8	90	PA, SA	French	[1]
Davison et al. (1998)	2.5	2	79	PA, SA (FSH> 20IU/l)	English	[16]
Castillo et al. (1992)	32.0	15	47	POI	Chilean	[17]
Rebar and Connolly (1990)	25.4	16	63	PA, SA	American	[18]

CA, chromosomal abnormalities; PA, primary amenorrhea; SA, secondary amenorrhea; EM, early menopause.
aIncluding two Swyer syndrome.

sporadic groups, which was higher than our result.

The development of new information related to etiology and the molecular basis of POI could contribute to understanding ovarian physiology, genetic counseling, and fertility guidance.

In conclusion, we found that the prevalence of genetic abnormalities in POI is 21.5%. Our study clearly demonstrates the association between chromosomal abnormalities and POI and underlines the essential role of the X chromosome in the etiology of POI. In light of our results, it could be recommended that cytogenetic investigation should routinely become a part of the management of women diagnosed with POI regardless of the patient's age and even when there are no other clinical features suggesting any chromosomal abnormality.

Conflict of interest

The authors declare no conflict of interest related to this work.

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